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Effect of Papaverine on Acetylcholinesterase in Rat Brain

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Abstract □ Papaverine administration produced significant increases in acetylcholinesterase activity in cerebral cortex and striatum of rat brain. Two related compounds, tetrahydropapaverine and tetrahydropapaveroline, also gave similar effects. However, their actions seem to be indirect because papaverine has no *in vitro* effect on the enzymatic activity.

Keywords □ acetylcholinesterase (AChE), papaverine, tetrahydropapaverine, tetrahydropapaveroline.

Acetylcholinesterase (AChE, EC 3.1.1.7.) plays a key role in cholinergic mechanisms of nerve transmission. Inhibition of AChE causes an accumulation of acetylcholine which appears to be responsible for the occurrence of convulsions and uncontrolled depolarization of cholinergic neurons at neuromuscular junctions. Intoxication and death from organophosphates such as diisopropylfluorophosphate (DFP),[1,2] soman,[2] and paraoxon[1] is thought to occur through irreversible inhibition of AChE[3].

The current therapy of the majority of organophosphate poisoning is administration of atropine and oxime. Atropine acts as a blocker at the muscarinic receptor and thus protects the receptor site from accumulated acetylcholine. The therapeutic effect of the oximes is based on their ability to reactivate the inhibited AChE[4-6]. The therapy may be hampered by a conversion of the inhibited enzyme into a non-reactivatable form. This process is based on dealkylation and proceeds rapidly when AChE is inhibited by soman, which is regarded as one of the potential chemical warfare agents. Therefore, the development of more effective antidotes for anticholinesterase toxicity has been tried.

In this investigation, we found that AChE activity of rat brain could be increased by papaverine and related compounds. It might provide another approach for the development of new antidote for the toxicity of anticholinesterase agents.

EXPERIMENTAL METHODS

Animals

Male Sprague-Dawley rats weighing about 180g were used. They were housed in cage with free access to laboratory chows and water, and maintained in the environment of constant temperature throughout the experiments.

Materials

Papaverine hydrochloride, tetrahydropapaverine hydrochloride, tetrahydropapaveroline hydrobromide, acetylthiocholine iodide, 5,5′-dithiobis(2-nitrobenzoic acid) [DTNB] were obtained from Sigma Chemical Co. Electric eel acetylcholinesterase as an enzyme standard and bovine serum albumin as a protein standard were also from Sigma Chemical Co. Other reagents used were of a first grade.

Administration of drugs

Papaverine, tetrahydropapaverine, and tetrahydropapaveroline were injected intraperitoneally once a day for one week or two weeks. Control group was injected with saline. The rats were sacrificed by decapitation 24 hours after the last injection of drugs. Brains were removed and dissected to obtain cerebral cortex and striatum.

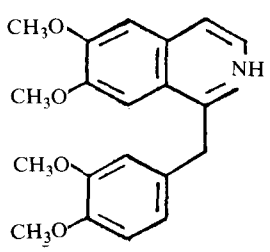
Preparation of homogenates

One per cent homogenate was prepared in 10 mM Na/K phosphate buffer (pH 7.4) using a glass homogenizer. Homogenates made were used for assay on the same day.

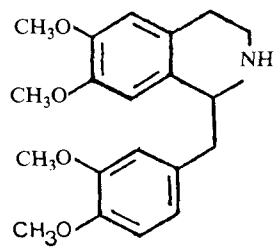
Protein concentrations were determined by the method of Lowry *et al.*[7] using bovine serum albumin as standard.

Acetylcholinesterase (AChE) assay

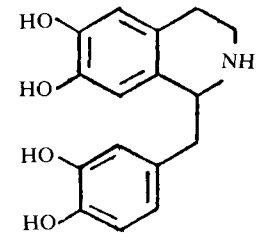
Acetylcholinesterase (AChE) activity of homo-



Papaverine



Tetrahydropapaverine



Tetrahydropapaveroline

genate was determined according to a slight modification of the procedure described by Ellman *et al.*[8] Acetylthiocholine was used as substrate and direct photometric determination of thiocholine with DTNB reagent was performed. Enzymatic activity was expressed as a change in absorbance ($\triangle OD_{410}$) per minute per mg protein. Results were expressed as mean ±S.E., and statistical significance of the differences of means were analyzed by Student's t-test.

In vitro effect of papaverine

In order to examine the *in vitro* effect of papaverine, AChE assay was done in the presence of papaverine at the concentration of 10^{-7}M, 10^{-6}M, 10^{-5}M, and 10^{-4}M. The homogenate of cerebral cortex and electric eel acetylcholinesterase were used as enzyme sources. AChE activity was expressed as a mean of three determinations.

RESULTS AND DISCUSSION

Papaverine is known to act directly on smooth muscle causing relaxation. Acetylcholinesterase activity of mouse brain was reported to seem to increase after repeated administration of papaverine[9]. However, experiments was not done to confirm this potentially important observation which might have implications for treating toxicity of anticholinesterase agents. We attempted to investigate this possibility using rats, and expanded to include two related compounds, tetrahydropapaverine and tetrahydropapaveroline.

In an initial experiment, daily administration of papaverine (10 mg/kg/day) for one week or two weeks produced significant increase in acetylcholinesterase activity of cerebral cortex and striatum (Table I). The increments did not differ meaningly in the two regions of rat brain. Also, it appeared that duration of administration (1 week or 2 weeks) could not make a difference in the increase of the enzymatic activity. Administration of papaverine did not give a change in the growth rate of rats (data not shown).

When additional doses of papaverine were included to establish a dose-response relationship, the

Table I. Effect of papaverine administration on acetylcholinesterase activity in rat brain

	Dose (mg/Kg/day, i.p.)		N[a]	AChE activity+ ($\triangle OD_{410}$/min/mg protein)	Relative activity (%)
Cerebral cortex					
1 week	Control		7	0.2093 ± 0.0150	100
	Papaverine	10.0	7	0.2456 ± 0.0132*	117
2 weeks	Control		7	0.1877 ± 0.0116	100
	Papaverine	10.0	7	0.2451 ± 0.0184*	121
Striatum					
1 week	Control		7	1.5973 ± 0.0392	100
	Papaverine	10.0	7	1.7849 ± 0.0416*	112
2 weeks	Control		7	1.4125 ± 0.0828	100
	Papaverine	10.0	7	1.5222 ± 0.0228	108

[a] Number of rats used.
+ Values of AChE activity are expressed as mean ± S.E.
* $p<0.05$

Table II. Effect of 7-day administration of various doses of papaverine on acetylcholinesterase activity in rat brain

	Dose (mg/Kg/day, i.p.)		N[a]	AChE activity+ (ΔOD_{410}/min/mg protein)	Relative activity (%)
Cerebral cortex					
	Control		7	0.1511 ± 0.0041	100
	Papaverine	2.5	7	0.1730 ± 0.0060*	115
	Papaverine	10.0	7	0.1854 ± 0.0066**	123
	Papaverine	40.0	7	0.1868 ± 0.0065**	124
Striatum					
	Control		6	1.2580 ± 0.0277	100
	Papaverine	2.5	6	1.3805 ± 0.0308*	110
	Papaverine	10.0	6	1.4704 ± 0.0336**	117
	Papaverine	40.0	6	1.5050 ± 0.0307**	120

[a] Number of rats used.
+Values of AChE activity are expressed as mean ± S.E.
*$p<0.05$
**$p<0.01$

increase in acetylcholinesterase activity of higher dosage group after one week administration was larger than that of lower dosage group in both cerebral cortex and striatum (Table II). But, there was not a linearity in the relation between doses of papaverine and increases in acetylcholinesterase activity of rat brain. On the other hand, some kind of limits in the increase were observed. It might suggest that the increase by papaverine could be indirect.

Our attempt was extended to test two congeners of papaverine, tetrahydropapaverine and tetrahydropapaveroline. Each of these substances caused a 20-35% increase in acetylcholinesterase activity of both cerebral cortex and striatum (Fig. 1). Tetrahydropapaverine gave a slightly higher increase. This result might suggest that an increase by papaverine and its congeners is mainly due to their common aromatic ring structure.

It was examined that papaverine could affect acetylcholinesterase activity in the homogenate of rat brain. This was done to know whether papaverine can activate acetylcholinesterase directly if it could enter brain. When papaverine was added to reaction mixture at a concentration of up to 10^{-4}M, it could not change the enzymatic activity of cerebral cortex (Table III). It was rather a little inhibitory at high concentration (10^{-4}M) of papaverine. This was also true with pure electric eel acetylcholinesterase (data not shown). This confirms that papaverine itself is not a direct positive regulator of acetylcholinesterase in rat brain.

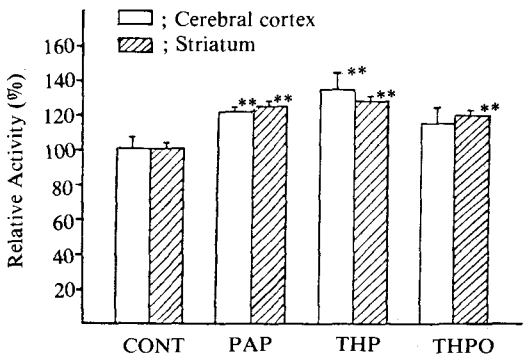


Fig. 1. Effect of 7-day administration of papaverine and its congeners on acetylcholinesterase activity in rat brain.
Each column represents the mean value obtained from seven rats. Vertical bars represent standard errors.
**$p<0.01$
CONT: control (saline)
PAP : papaverine (10 mg/kg/day, i.p.)
THP : tetrahydropapaverine (10 mg/Kg/day, i.p.)
THPO: tetrahydropapaveroline (10 mg/Kg/day, i.p.)

Papaverine has a variety of effects on smooth muscle which result in relaxation and inhibition of responses to some excitatory agents. One approach to the analysis of papaverine action involves cyclic nucleotides. There are some evidence that papaverine inhibits cyclic nucleotide phosphodiesterase ac-

Table III. ***In vitro*** **effect of papaverine on acetylcholinesterase activity in the homogenate of rat brain.**

Papaverine concentration (M)	AChE activity+ ($\triangle OD_{410}$/min)
0	0.040
10^{-7}	0.039
10^{-6}	0.039
10^{-5}	0.039
10^{-4}	0.038

+Values are expressed as a mean of three determinations.

tivity in some muscles implicating a rise in cellular cyclic AMP concentration as the mediator of relaxation[10-13]. It was also demonstrated that guanylate cyclases from various rat tissues can be inhibited by papaverine[14]. Also, there are several reports concerning the effects of tetrahydropapaveroline on monoamine metabolism in brain *in vivo*[15-17]. However, it is not known whether these findings correlate with papaverine action on acetylcholinesterase activity in rat brain. It requires further study. So far our study suggests that papaverine and its congeners can increase acetylcholinesterase activity in rat brain indirectly. Their exact action mechanism can not be defined at present. This type of future study may provide another approach for combating toxicity of anticholinesterase agents.

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